37th Plant Development Workshop

McMaster University

November 1, 2003

ROYAL BOTANICAL GARDENS

Royal Botanical Gardens Centre, Burlington

Plant Development Workshop

November 1, 2003

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8.15 - 9.00	Registration and poster setup
9.00	Brief welcome by John Lott
9.01 - 9.15	David Galbraith, **Royal Botanical Gardens Programs**
9.15 - 9.30	Dan Riggs, Siddiqui, Najeeb, Hasenkampf, Clare and Dengler, Ron. Department of Botany, University of Toronto. **Defects in chromatin condensation alter meristem cell differentiation programs.**
9.30 - 9.45	Jaideep Mathur, Hulskamp, Martin[1] and Berleth, Thomas. Department of Botany, University of Toronto, [1]Botanical Institute III, University of Koln. **Intracellular behavior and role of a microtubule end-binding protein *AtEB1-1* in plant cell morphogenesis.**
9.45 - 10.00	Arunika Guanawardena.[a,b], Greenwood, John S.[c], and Dengler, Nancy G.[a], [a]Department of Botany, University of Toronto, [b]Department of Agriculture and Biology, Faculty of Agriculture, University of Peradeniya, Sri Lanka, [c]Department of Botany, University of Guelph. **Programmed cell death remodels lace plant leaf shape during development.**
10.00 - 10.15	Shengwu Ma. Transplantation Immunology Group, London Health Sciences Centre and Department of Biology, University of Western Ontario. **Production of transgenic plants expressing human glutamic acid decarboxylase (hGAD 65) and murine interleukin (IL)-4 for oral immunontherapy of autoimmune diabetes.**
10.15 - 10.50	Coffee break and viewing posters
10.50 - 11.05	Sarah Rosloski, Poduska, Branislava and Grbic, Vojislava. Department of Biology, University of Western Ontario. **Role of floral pathway integrators in the late flowering, *Aerial Rosette* phenotype of *Arabidopsis thaliana* accession Sy-0.**
11.05 - 11.20	Qing Wang, Poduska, Branislava, Humphrey, Tania, Redweik, Antje and Grbic, Vojislava. Department of Biology, University Western Ontario. **Characterization of a novel flowering time control locus *Aerial Rosette 1.***

11.20 - 11.35 Yashwanti Mudgil[1], Salt, J.[1], Stone, S.L.[2], and Goring D.R.[1] [1]Department of Botany, University of Toronto, [2]Department of Ecology and Evolution, University of Chicago.
Functional characterization of the Arc1-related (*At*pub) family in *Arabidopsis*.

11.35 - 11.50 Yosr Haffani, Silva, Nancy F., Keatley, Sarah, Aldea, May G., and Goring, Daphane R. Botany Department, University of Toronto.
Over-expression and antisense-suppression of the Perk1 receptor kinase in *Arabidopsis* leads to changes in growth and seed production.

11.50 - 12.05 Fengshan Ma[1,2], Peterson, Carol A.[1], Gijzen, Mark[2] [1]Department of Biology, University of Waterloo, [2]Southern Plant Protection and Food Research Centre, Agriculture and Agri-Food Canada.
Cuticle and surface deposits on soybean seeds: structure and possible functions.

12.05 - 12.20 Nadia Talent and Dickinson, T.A. Centre for Biodiversity and Conservation Biology, Royal Ontario Museum and Department of Botany, University of Toronto.
Polyploidy of embryo and endosperm in sexual and apomictic Crataegus: data from flow cytometry.

12.20 - 2.20 Lunch , Poster Presentations, Mediterranean Garden

2.20 - 2.35 Ewa Cholewa[1], Smith, Don[2], and McIver, John[1] [1]BiosAgriculture, Ste-Anne-de-Bellevue, Quebec, [2]Departmen of Plant Science, McGill University.
Application of lipo-chitooligosaccharides to soybean leaves stimulates and inhibits photosynthesis at high and low light intensities respectively.

2.35 - 3.05 Larry Peterson. Department of Botany, University of Guelph.
Effect of mycorrhizal fungi on host cell cytoskeleton.

3.05 - 3.25 Robin K. Cameron. Department of Biology, McMaster University
Who's influencing whom? Environmental and developmental effects on plant defense responses.

3.25 - 4.00 Coffee break and Poster Presentations

4.00 - 4.30 Elizabeth Weretilnyk, Guevara, D.R., Charlton, E., Lott, J.N.A., McCarry, B.E., and Golding, G.B. Department of Biology, McMaster University, Hamilton, ON L8S 4K1. ***Tellungiella:*** **A model plant for studying osmotic stress tolerance in plants.**

4.30 - 5.00 Marilyn Griffith, University of Waterloo.
Development of winter rye at cold temperatures: the regulation and role of antifreeze proteins.

Oral Presentation Abstracts

Riggs, Dan, Siddiqui, Najeeb, Hasenkampf,Clare and Dengler, Ron. Department of Botany, University of Toronto. **Defects in chromatin condensation alter meristem cell differentiation programs.**

During the cell cycle, chromosome structure dramatically changes from an interphase configuration, wherein the chromatin is decondensed and active in replication and/or transcription, to a mitotic configuration where compaction precludes many information transfer processes. Entangled fibers are resolved into physically discrete entities which can be easily distinguished by light microscopy. Condensation is mediated in part by a conserved complex of 5 proteins, and we have identified mutations in three SMC components of the 'condensin complex' in *Arabidopsis thaliana.* Loss of condensin function, either by mutation or by antisense technology, can be correlated with defects in meristem organization and function and in some situations, gametogenesis and/or embryogenesis are compromised. We will present a summary of our past, present and future work in this area.

Mathur, Jaideep, Hulskamp, Martin[1]and Berleth, Thomas. Department of Botany, University of Toronto, [1]Botanical Institute III, University of Koln. **Intracellular behavior and role of a microtubule end-binding protein *AtEB1-1* in plant cell morphogenesis.**

A group fo MAPs (microtubule-associated proteins) called +TIPs (plus-end tracking proteins) include proteins of the EB1 (End-Binding) family and have been shown to specifically label growing microtubule-ends in diverse organisms. The EB1-family proteins share a common N-terminal type-2 Calponin Homology (CH) domain and certain conserved leucine residues that suggest a basic domain-leucine zipper (bZIP) motif. The C-terminal regions of the different EB1 proteins are move divergent. *Arabidopsis* possesses three EB1-like genes of which *AtEB1-1* differs from all known EB1 proteins in having a very long acidic C-terminal tail comprising of glutamic-acid residues. Further, in marked contrast to other EB1 proteins the GFP-AtEB1-1 fusion protein localizes not only to microtubule plus-ends but also to motile, pleiomorphic tubulo-vesicular membrane networks that surround other organelles and frequently merge with the endoplasmic reticulum. AtEB1-1 behavior thus resembles that of +TIPs such as the Cytoplasmic Linker Protein CLIP-170 for which homologs have not been identified in plants. CLIP-like proteins associate with and, pull-along membrane-tubules. We shall present observations obtained from wild-type and mutant *Arabidopsis* plants carrying the *GFP-AtEB1-1* transgene that elaborate upon the behavior and interactions of microtubules with endo-membranes and the actin cytoskeleton. These observations enhance our comprehension of intracellular workings during polarized cell morphogenesis in higher plants.

Reference: Mathur et al. Current Biology 2003 (in press)

Guanawardena[a,b], Arunika N., Greenwood, John S.[c] and Dengler[a], Nancy G. [a] Department of Botany, University of Toronto. Department of Agricultural Biology, Faculty of Agriculture, University of Peradeniya, Sri Lanka [c] Department of Botany, University of Guelph, Guelph. **Programmed cell death remodels lace plant leaf shape during development.**

PCD (programmed cell death) functions in the developmental remodeling of leaf shape in higher plants, a process analogous to digit formation in the vertebrate limb. In this study we provide a cytological characterization of the time course of events as PCD remodels young expanding leaves of the lace plant. Tonoplast rupture is the first PCD event in this system indicated by alterations in cytoplasmic streaming, loss of anthocyanin color, and ultrastructural appearance. Nuclei become TUNEL-positive shortly afterwards, but do not become morphologically altered until late stages of PCD. Genomic DNA is fragmented, but not into internucleosomal units. Other cytoplasmic changes, such as shrinkage and degradation of organelles, occur later. This form of PCD resembles tracheary element differentiation in cytological execution, but requires unique developmental regulation so that discrete panels of tissue located equidistantly between veins undergo PCD, while surrounding cells do not.

Ma, Shengwu. Transplantation Immunology Group, London Health Sciences Centre and Department of Biology, University of Western Ontario. **Production of transgenic plants expressing human glutamic acid decarboxylase (hGAD 65) and murine interleukin (IL)-4 for oral immunontherapy of autoimmune diabetes.**

The induction of immunological unresponsiveness by feeding soluble antigens, termed oral tolerance, has been attracting attention as a potential immunotherapy for autoimmune disease. However, as oral tolerance induction requires ingestion of large amounts of protein antigens, the clinical use of oral tolerance induction as a therapy may be critically dependent on the availability of large amounts of relevant protein antigens at low cost and the establishment of a simple and effective antigen delivery system. Recently we demonstrated that transgenic plants could be used as an approach of combining an economic production method with an oral delivery system for a diabetes-associated autoantigen, mouse GAD67, to induce oral tolerance to prevent autoimmune diabetes in non-obese diabetic (NOD) mouse serving as a murine model of human diabetes. Use of adjuvants may enhance oral tolerance, but commonly used mucosal adjuvants such as cholera toxin B subunit (CTB) may be limited by neutralizing immune responses. Interleukin-r (IL-4) has multiple immunoregulatory properties including the downregulation of immune responses, and can potentially act as a mucosal adjuvant. In this study transgenic plants expressing human GAD65 and murine IL-4 were produced. NOD mice, when given combined IL-4 and GAD65 plant tissue, were protected from the development of diabetes, while those receiving IL-4 or hGAD65 plant tissue alone were not protected, suggesting that IL-4 and GAD65 expressed by transgenic plants can induce protective oral immune tolerance and is synergistic in preventing diabetes. This novel approach may be useful in the treatment of human diabetes.

Rosloski, Sarah, Poduska, Branislava and Grbic, Vojislava. Department of Biology, University of Western Ontario. **Role of floral pathway integrators in the late flowering, *Aerial Rosette* phenotype of *Arabidopsis thaliana* accession Sy-0.**

The Sy-0 accession of *Arabidopsis thaliana* is being investigated to understand the role of flowering-related genes in plant morphology. Sy-0 is characterized by a late-flowering time, development of leafy rosettes in the axils of cauline leaves and reversion of inflorescences and flowers. Mapping has identified three genes, *AERIAL ROSETTE1* (*ART1*), *FRIDGIDA (FRI)* and *FLOWERING LOCUS C (FLC)*, that contribute to this heterochronic shift in meristem development. *ART1* and *FRI* interact to strongly increase expression of *FLC*, a known repressor of flowering. Our data suggest that *ART1*, *FRI* and *FLC* modulate the expression of floral pathway integrators (*FPIs*), which may promote the late flowering phenotype. Future work will focus on the specific roles *ART*, *FRI* and *FLC* have in the modulation of FPI expression and the association of these effects with the aerial rosette phenotype.

Wang, Qing, Poduska, Branislava, Humphrey, Tania, Redweik, Antje and Grbic, Vojislava. Department of Biology, University of Western Ontario. **Characterization of a novel flowering time control locus *AERIAL ROSETTE 1*.**

Plant flowering time is controlled by various environmental and developmental cues. In order to understand the mechanisms that control the transition from vegetative to reproductive growth, we are investigating an *Arabidopsis* ecotype Sy-0. This ecotype has delayed flowering time under inductive conditions, leading to dramatical increase in numbers of rosette leaves. This phenotype requires interaction of three loci, *AERIAL ROSETTE 1 (ART1), FRIGIDA (FRI)* and *FLOWERING LOCUS (FLC)*. The novel flowering locus *ART1* was mapped to 14 cM proximal to FLC on chromosome 5, and located in ~16 kb of a genomic fragment. This region was sequenced. A likely candidate, the putative transcriptional factor *HUA2* gene, was identified. Two amino acid substitutions were found in the *ART1* allele as compared to the *HUA2* Columbia allele. About 10 kb of the *ART1* genomic clone was used for the complementation analysis. Meanwhile, one T-DNA line with the insertion in the 2nd exon of the *HUA2* gene was obtained. The *HUA2* transcript was not detectable in this T-DNA line. It is likely a null allele of *HUA2*. The flowering time as slightly early in this line. The further functional analysis of the *ART1* allele is underway.

Mudgil, Yashwanti[1], Salt, Jennifer.[1], Stone, S.L.[1], Shiu, S.H.[2], and Goring, D.R.[1]1Department of Botany, University of Toronto, [2]Department of Ecology and Evolution, University of Chicago. **Functional Characterization of the Arc1-Related (*At*pub) Family in *Arabidopsis*.**

Arabidopsis contains a large number of U-box proteins (*At*PUBs) with many of these showing sequence similarity to *Brassica* ARC1. This amino acid similarity is found in the U-box region followed by several arm repeats. *Brassica* ARC1 is an E3 ubiquitin ligase which act downstream of the S Receptor Kinase (SRK) to promote ubiquitination and protein degradation which in turn results in the rejection of self-incompatible *Brassica* pollen. As part of the characterization of the ARC1 related *At*PUBs, we have developed better algorithms to detect plant arm repeats and used these to identify arm repeat proteins in *Arabidopsis*. Phylogenetic analysis showed that the 108 predicted *Arabidopsis* arm repeat proteins can be divided into multiple clusters with wide differences in their domain organizations. Interestingly, 41 of the 108 *Arabidopsis* arm repeat proteins belong to the U-box *At*PUB family and represent the largest class of *Arabidopsis* arm repeat proteins. We are interested in understanding the function of these ARC1 related *At*PUB proteins in self-compatible *Arabidopsis*. Based on a phylogenic tree, representative members were selected from each subgroup for detailed analyses. Using RNA blot analysis, we have demonstrated that they are expressed in a variety of tissues in *Arabidopsis*. In addition, the selected *At*PUBs have been found to be functional E3 ubiquitin ligases. Finally, the yeast two-hybrid system has been used to analyze the interactions between the selected *At*PUBs and various *Arabidopsis* receptor kinases to determine if similar interactions to that of SRK and ARC1 exist in *Arabidopsis*. Our results indicate that the *At*PUBs interact specifically with S-domain receptor kinases. Thus, the ARC1 related *At*PUBs may be involved in a conserved receptor kinase signaling pathway regulating other aspects of plant growth and development.

Haffani, Yosr Z., Silva, Nancy F., Keatley, Sarah, Aldea, May G., and Goring, Daphne R. Botany Department, University of Toronto. **Over-expression and antisense-suppression of the Perk1 receptor kinase in *Arabidopsis* leads to changes in growth and seed production.**

The PERK receptor kinase family is characterized by an extracellular domain that is proline-rich and extensin-like followed by a transmembrane domain and kinase domain. The original member, PERK1, was isolated from *Brassica napus*, and 14 PERK1-related members were subsequently identified in the *Arabidopsis* genome. One *At*PERK member was readily identifiable as the orthologue of *Bn*PERK1 based on high similarities in sequence identity, domain organization, and expression patterns. Overexpression and antisense suppression experiments were preformed using the *Bn*PERK1 cDNA under the control of the 35S CaMV promoter and introduced into *Arabidopsis thaliana* Col-0. In the case of the antisense suppression, the *Bn*PERK1 cDNA shared sufficient sequence similarity to potentially suppress all 14 *At*PERK members. In both sets of transgenic *Arabidopsis*, several heritable changes in growth and development were observed. The overexpressing PERK1 *Arabidopsis* plants showed increases in height, secondary branching, root growth and seed production as well as loss of seed dormancy when compared to the wild-type *Arabidopsis* Col-0 plants. The PERK1 antisense suppressing transgenic plants showed various defects ranging from lethal phenotype with seedlings completely lacking roots to a less severe phenotype with loss of apical dominance and partial sterility compared to the wild-type *Arabidopsis* Col-0 plants. We are currently screening for T-DNA insertions in individual *At*PERK members to examine the individual contributions of these genes to the observed phenotypes.

Ma, Fengshan[1,2], Peterson, Carol A.[1] and Gijzen, Mark[2]. [1]Department of Biology, University of Waterloo, [2]Southern Plant Protection and Food Research Centre, Agriculture and Agri-Food Canada, London.. **Cuticle and surface deposits on soybean seeds: structure and possible functions.**

There are two types of soybean seeds, highly permeable (normal) and sparingly permeable in terms of water permeability. This property is determined by the structure of the palisade layer (epidermis) in the seed coat. There is a cuticle of about 1 μm in thickness on the palisade layer. This cuticle exhibits a regulate pattern and is continuous all over the extrahilar region. Permeable and impermeable cultivars differ in the physical properties of their cuticles. A highly permeable seed has a weak cuticle that tends to crack so that imbibition readily occurs. A sparingly permeable seed has a strong cuticle that does not break easily. In all cultivars, the cuticle is covered by surface deposits on varied areas and with different patterns, depending on the cultivar. There are three types of surface deposits: 1) a thin layer of amorphous material, 2) accumulations of amorphous and crystalline substances, and 3) fragments of endocarp tissues. The origins of types 1 and 2 deposits are not obvious. The deposits *per se* may not increase the hardedness of the seed coat, but may protect the underlying cuticle from breaking. Characterization of the chemical compositions of the surface deposits will provide further information concerning their possible contribution to the control of water entry.

Talent, Nadia and Dickinson, Tim A. Centre for Biodiversity and Conservation Biology, Royal Ontario Museum, Toronto and Department of Botany, University of Toronto. **Polyploidy of embryo and endosperm in sexual and apomictic Crataegus: data from flow cytometry.**

Although diploid plants of Crataegus are wholly or largely sexual, many polyploids (triploids, tetraploids, and occasional aneuploids) are apomictic. Parthenogenesis in an unreduced, 8-nucleate megagmetophyte gives rise to a matroclinous embryo. Pollination is required for seed formation but is unknown how the usual requirement for balanced maternal and paternal genetic contributions to the endospearm is overcome. We have surveyed the DNA amounts in leaft tissue from many trees by flow cytometry and have found that the different ploidy levels are distinguishable, but we have yet to find a plant with ploidy higher than 4x. We are now applying this technique to pollen and to mature and developing seeds. Pollen DNA measurements from diploids and tetraploids match the 2C value of the parent, a result that is consistent with meiotic reduction and a binucleate pollen grain, as is seen in Rosaccae generally. Diploid plants produce seeds with triploid endosperm. Pollen from a tetraploid on diploid plants produces triploid seeds that have tetraploid endosperm. The reciprocal pollination appears to result in only tetraploid embryos. Seed from open-pollinated tetraploids showed approximately 10x DNA amounts in the endosperm, with higher values in some seeds. We tentatively interpret these DNA amounts as resulting from the involvement of two maternal nuclei and a single sperm nucleus. Our results have implications regarding seed formation in male-sterile triploids, the origin of triploid Crataegus generally, and non-conspecific sexual reproduction through pollen by tetraploids.

Cholewa, Ewa[1], Smith, Don[2], and McIver, John[1]. [1]BiosAgriculture, Ste-Anne-de-Bellevue, Montreal, [2]Department of Plant Science, McGill University. **Application of lipo-chitooligosaccharides to soybean leaves stimulates and inhibits photosynthesis at high and low light intensities respectively.**

Lipo-chitooligosaccharides (LCOs) are powerful developmental regulators produced by rhizobia in response to legume-roots-secreted flavonoids. These very potent amphiphilic molecules set in motion a whole range of plant responses that eventually are manifested in organogenesis and formation of the nodule in symbiotic legumes. We are exploring the potency and efficacy of LCO application as a plant-growth enhancing factor in commercially important fruits and vegetables. Results of earlier studies indicated that foliar application of LCOs is beneficial to number of host and non-host plant species and results in increases in the photosynthetic rates. In this study we investigated whether those increases are invoked in the LCO-treated leaves only. LCO application to one soybean leaf resulted in an increase in photosynthesis (measured as CO_2 uptake) in that leaf 24 hours after application. However, immediate measurements (2 hours after LCOs application) revealed that there is a slight decrease in the photosynthetic rate in the treated leaf as compared to the untreated leaf on the same plant or to control plants. Such decreases were observed 3 times during the course (96 h) of experiment and were correlated with low light intensity during cloudy, winter days in the greenhouse. During sunny days, when light intensity was high, the photosynthetic rates in the LCO treated leaf were the same as in the non-treated leaf. These averaged 21% higher than the rates in separate control plants. This indicates a systemic translocation of LCOs (and/or its induced signal) from leaf to leaf within plant. Our findings indicate a dual LCOs role in photosynthesis: causing increases in photosynthetic rates at high light intensities and further decreases at low light intensities. The mechanism of LCOs regulation of photosynthesis is unknown and requires further investigation.

Peterson, R. Larry. Department of Botany, University of Guelph. **Effect of mycorrhizal fungi on host cell cytoskeleton.**

Mycorrhizal fungi alter root development at the organ, cellular, and sub-cellular levels. Since nutrient exchange between the symbionts occurs at the cellular level, there has been considerable interest in determining the alterations in root cell cytology as roots become colonized by mycorrhizal fungi. The nutrient exchange interface in most mycorrhizal categories occurs between fungal hyphae that breach the plant cell wall but are enclosed by host cell-derived perifungal membrane and interfacial matrix material deposited between this membrane and the fungal cell wall. The interfacial matrix acts as an apoplastic compartment through which nutrients must pass. In ectomycorrhizas, however, fungal hyphae remain external to the plant cell and form an intercellular Hartig net. Nutrients, therefore, are exchanged external to the plant cell plasma membrane. One of the questions of interest is the fate of the cytoskeleton microtubules (MTs) and actin filaments (AFs) during the colonization process, and potential roles it might play in the establishment of the exchange interface. Previous research with arbuscular mycorrhizas and orchid mycorrhizas has shown marked changes in the organization of the cytoskeleton, with a close association between both host MTs and AFs with intracellular hyphae. Recently, we have shown similar changes in MTs in ectendomycorrhizas and monotropoid mycorrhizas. Also, we have re-examined ectomycorrhizas since earlier reports indicated that MTs and AFs were lost from root cells as the Hartig net develops. Our results do not support these observations in that MTs remain in cells adjacent to Hartig net hyphae.

Cameron, Robin K., McMaster University, Department of Biology **Who's influencing whom? Environmental and developmental effects on plant defense responses.**

Plants are hosts of many types of pathogens including bacteria, viruses and fungi. In many instances plants recognize that they are being attached quickly and mount a successful defense response. Plants possess a number of defense responses including specific Resistance gene receptors that recognize specific avirulence proteins produced by certain pathogen races and an unknown number of receptors which recognize common pathogen molecules (e.g. FLS2 receptor which recognizes bacterial flagellin). Other defense responses in plants include Systemic Acquired Resistance (SAR) and Age-related resistance (ARR). What all these responses have in common is that environmental as well as developmental factors can affect the timing and/or outcome of the response. A few examples of how, who or what influences plant defense will be discussed, along with other hot topics in the plant-pathogen interaction field.

Weretilnyk, EA., Guevara, D.R., Charlton, E., Lott, J.N.A., McCarry, B.E., and Golding, G.B.
Department of Biology, McMaster University. ***Thellungiella*: A model plant for studying osmotic stress tolerance in plants.**

Thellungiella salsuginea Pall. is an annual or biennial diploid (2n = 14) crucifer plant that grows on saline shores and alkaline or saline flats and prairies. Its natural distribution extends from the Yukon, southwest to British Columbia, and across the Prairie Provinces to western Ontario. Its capacity to survive and grow in cold areas under drought or saline conditions indicates that *Thellungiella* is a powerful model to identify developmental, biochemical, and physiological traits underlying environmental stress tolerance in plants. Comparisons made between genes sequenced in this plant and its close relative, *Arabidopsis thaliana*, show as little as 5% variation in the genetic make-up between these two species. While Arabidopsis is an ideal genetic model for many studies, it shows little tolerance to drought, freezing temperatures or salinity. *Thellungiella*, however, offers exceptional potential as an abiotic stress model organism with the added advantage of being genetically similar enough to *Arabidopsis* for us to exploit the abundant genetic resources (including a completely sequenced genome), tools and knowledge amassed for *Arabidopsis* to identify mechanisms underlying stress tolerance in plants.

Griffith, Marilyn, University of Waterloo. **Development of winter rye at cold temperatures: the regulation and role of antifreeze proteins.**

Freezing-tolerant plants survive the formation of extracellular ice in their tissues when they are exposed to subzero temperatures. Winter rye, an overwintering cereal, becomes maximally freezing-tolerant only after it grows new leaves at cold temperature. During development in the cold, winter rye plant secrete antifreeze proteins that modify the freezing process. These proteins are also pathogenesis-related proteins and form oligomeric complexes of glucanases, chitinases, thaumatin-like proteins and lipid-transfer proteins.

Poster Presentation Abstracts

Beaton, Laura and Dudley, Susan, Department of Biology, McMaster University. **Salt and Manganese tolerance in a common roadside plant *Dispsacus sylvestris.***

Roadside soils are contaminated with a variety of substances toxic to plants, including manganese (Mn^{++}) from the use of the anti-knocking agent methylcyclopentadienyl manganese tricarbonyl (MMT) in gasoline, and salt from the use of de-icing salts. We investigated whether roadside populations of *Dipsacus sylvestris* Huds. were more tolerant of these contaminants during germination than were populations located in the more benign environment, old fields. We used field collected maternal families because the life history of the species, a monocarpic perennial, prevented us from growing the plants for one generation in a common environment. Family responses to the treatments are therefore a combination of genetic traits and maternal environmental effects. Consequently, we measured several traits likely to be determined by the maternal environment, including seed size and the quantity of sodium ions (Na^{+}) leached from seeds during germination. Roadside populations did not demonstrate any adaptation to elevated Mn^{++} levels during germination but did display greater tolerance to elevated salinity levels. Some families even showed enhanced growth in the presence of salt. Salt tolerance in roadside populations of *D. sylvestris* appeared to be highly related to both seed size and Na^{+} concentration of the seed leachate. Consequently, the observed salt tolerance of roadside *D. sylvestris* seeds may be a maternal environmental effect rather than a genetic trait

Bianchi, Andrea E.[1], Cornell, Kenneth A.[2], and Moffatt, Barbara A.[1], [1]Department of Biology, University of Waterloo, [2]Department of Biochemistry and Molecular Biology, Division of Hematology and Oncology, Oregon Health Sciences University. **Polyamines and plant development: an investigation of Methylthioadenosine nucleosidase activity in *Arabidopsis thaliana.***

Polyamines are ubiquitous compounds that have been associate with a wide range of developmental processes such as cell division, seed germination, flower development, fruit ripening and stress responses, although their exact physiological functions(s) are as yet unknown. One byproduct of polyamine synthesis is methylthioadenosine (MTA), which is catabolized by MTA nucleosidase into 5'-methylthioribose and adenine. Very little is known about this enzyme activity in plants. This project is using molecular and biochemical methods to investigate the role of this enzyme in *Arabidopsis thaliana.* The first part of this research involved identifying the genes encoding MTA nucleosidase within the genome of *Arabidopsis* using bioinformatic methods. Two such genes were identified, and were designated MTA1 and MTA2. MTA1 has been expressed in *E. coli*, and found to possess significant MTA nucleosidase activity. We next examined the expression patterns of each MTA gene in various organs using semi-quantitative RT-PCR. Finally, two strategies are being employed to investigate the effects of MTA nucelosidase deficiency in plants. *Arabidopsis* transformants that carry a T-DNA insert in either MTA1 or MTA2 were obtained from the SALK collection, and homozygotes were identified. Interfering RNA is also being utilized to study the effect of reducing the expression of both genes of plant development.

Chow, Brenda, Cutler, Sean and McCourt, Peter. Department of Botany, University of Toronto. **Analysis of ABA insensitive mutants expressing random GFP:DNA fusions.**

The plant hormone abscisic acid (ABA) has a key role in regulating seed dormancy and germination. Generally two types of genetic screens have been employed to dissect the signaling pathway of this response. These involve screening for germination mutants that are either ABA-insensitive (*abi*) or have an enhanced response to ABA (*era*). Most mutations affecting ABA sensitivity in germination have been identified in the Columbia ecotype and are genetically recessive; only two dominant mutations have been identified to date. These dominant mutations are in the Landsberg erecta ecotype. In an attempt to identify new genes regulating the ABA response in germination, we have initiated a screen for ABA-insensitive mutants in plants expressing random GFP:DNA fusions (Columbia ecotype). These fusions were generated from genomic DNA as well as mRNA isolated from etiolated seedlings and callus tissue. This approach can potentially identify new dominant mutations that may help in understanding the role of ABA in germination. We present the characterization of mutants identified in this screen.

Enstone, Daryl and Peterson, Carol. Department of Biology, University of Waterloo. **Hypoxia and ethylene effects on the anatomy of hydroponically grown maize roots.**

The stimulatory effects of both hypoxia and ethylene on aerenchyma development in roots are well documented. The suberized root exodermis is now considered to be the prime component of the radial oxygen loss (ROL) barrier, preventing oxygen diffusion from the aerenchyma to the rhizosphere. Does exodermal development also respond similarly to hypoxia and ethylene? We utilized maize (*Zea mays* L. cv. Seneca Horizon) seedlings growing in hydroponics to investigate this question. In one set of experiments, hypoxia was induced by adding agar to the non-aerated medium, creating a stagnant environment. In another experiment, ethylene was introduced into the aerating gas stream. Endodermal and exodermal suberin lamellae formation was quantitatively assessed in various root zones. Hypoxia increased exodermal suberin lamella deposition and reduced endodermal suberin lamella development compared to aerated controls. Hypoxia also induced large amounts of aerenchyma. Aerenchyma formation was not dependent upon exodermis maturation; however, when both lacunae and lamellae were present, they were usually radially aligned. Typically, the cortex adjacent to unemerged lateral roots lacked both aerenchyma and exodermal suberin lamellae. Applied ethylene stimulated enlargement of the cortex, and induced some aerenchyma formation. As under hypoxia, endodermal suberin lamella development was reduced. Ethylene, at the levels applied, produced no consistent effect on the exodermis. Suberin lamella reduction in the endodermis resulting from either hypoxia or applied ethylene treatments may enhance stelar aeration by reducing the resistance to oxygen diffusion from the cortical lacunae across the endodermis. We are continuing to examine the factors controlling exodermal development under hypoxic conditions.

Guevara, David R.[1], Charlton, E[1], McCarry, Brian E.[2], Dudley, Susan A.[1], Lott, John N.[1], Weretilnyk, Elizabeth A.[1]. Departments of Biology[1], Chemistry[2] McMaster University. **Morphological, physiological and biochemical characterization of *Tellungiella salsuginea* plants in response to osmotic stress.**

Thellungiella salsuginea is a subarctic plant that is highly tolerant to salt, drought and freezing compared to its close relative, *Arabidopsis thaliana*. *T. salsuginea* was gradually salt-stressed to a final level of 300 mM NaCl or subjected to drought. Salinization led to a drop in leaf water and solute potentials relative to controls (unstressed); plants remained turgid, an indication of osmotic adjustment. Rates of stomatal conductance, transpiration and photosynthesis decreased to approximately half those for controls. Leaves of salt-stressed plants accumulate red pigments in the sub-epidermal layer but otherwise resemble leaves of control plants with respect to many morphological features including stomatal number. With drought, plants showing a leaf relative water content (RWC) above appoximately 35% were able to rehydrate upon rewatering; plants with leaf RWCs below this level survived by growth of new leaves and not rehydration of existing ones. Using gas chromatography/mass spectrometry (GC/MS) we have identified a number of significant qualitative and quantitative differences between metabolites present in leaves of osmotically stressed plants compared to controls growing under otherwise identical conditions and the profile for metabolites in salt-stressed plants is distinct from that of plants exposed to drought. Identification of the metabolites appearing under stress is currently being done in order to determine which solute(s) contribute towards osmotic adjustment under saline conditions.

Kang, Julie and Dengler, Nancy G, Department of Botany, University of Toronto. **Cell cycling frequency and expression of the homeobox gene *AtHB-8* during leaf vein development in *Arabidopsis*.**

The histogenetic aspects of plant development depend on regulation of cell division plane, timing, and frequency to produce cell units of correct size and shape for mature function. Differences among the dermal, ground and vascular tissue systems arise during development, largely through regulation of these aspects of cell cycling in relation to overall tissue expansion. Using a *cyclin1At::GUS* reporter construct, we demonstrate quantitative differences in cell cycling frequency among tissue systems and among primary, secondary and tertiary veins; these differences are superimposed upon a more general longitudinal gradient of cell division frequency in developing leaves of *Arabidopsis*. Patterns of cell cycling frequency coincide almost exactly with those of the earliest known molecular marker of procambial identity, the HD-ZIP class III homebox gene *AtHB-8*, suggesting that *AtHB-8* may play a role in regulating the early events of procambial development, including procambium-specific patterns of cell cycling.

Krogan, Naden and Berleth, Thomas. Department of Botany, University of Toronto. **Transcriptional regulation by the Auxin Response Factor *MONOPTEROS*.**

The Arabidopsis *MONOPTEROS (MP)* gene plays a central role in embryo axis and vascular strand formation. *MP* encodes a transcription factor of the 'Auxin Response Factor' (ARF) family, whose members regulate the expression of auxin-induced genes by binding to conserved promoter 'Auxin Response Elements' (AuxREs). Several lines of evidence have implicated auxin in cell pattern formation. *MP* could therefore relay auxin signals in embryo axis patterning and vascular differentiation. Its target genes, however, are unknown. Transcriptional targets of *MP* likely include genes involved in vascular development and auxin signaling. To search for such direct downstream targets, a *mp* mutant line has been generated containing a posttranslationally inducible *MP* transgene (employing the hormone binding domain of the glucocorticoid receptor). This *MP:GR* transgene successfully and reversibly rescues the *mp* mutant, providing a suitable tool for elucidating direct targets. Promising targets revealed from this inducible system include the HD-ZIP III gene *ATHB-8*, the putative auxin efflux carrier *PIN1*, and auxin inducible *IAA* genes. These results raise interesting implications in terms of mechanisms of vascular pattern formation and auxin signaling in general.

Liu, Jessica C., Ockenden, Irene, Truax, Michael and Lott, John N.A. Department of Biology, McMaster University. ***Low phytic acid* rice grains: structure and mineral nutrients.**

The main mineral nutrient storage compound in grains is phytate, a salt of the effective chelator called phytic acid (PA). Phytic acid is not broken down in the digestive tract of monogastic animals including humans, poultry and swine and thus reduces bioavailability of Fe, Zn and Ca. Phytate in manure also contributes to phosphate pollution of waterways. This study describes differences and similarities between normal Kaybonnett rice grains and grains of *low phytic acid* (*lpa* 1 - 1) mutant in which the PA concentration is reduced about 45% while the total phosphorus concentration remains about normal. Electron microscopy of aleurone layer cells revealed that protein bodies in the normal grain had larger diameter phytate globiods than in the *lpa* grains, which had numerous smaller globiods. Reduction in globoid size/volume is consistent with the PA reduction in the *lpa* grains. Energy dispersive x-ray analysis showed that the globoids in aleurone cells in both types of dry grains contained mainly P, K and Mg with occasional traces of Ca, Mn, Fe, or Zn. The *lpa* mutant did not shift mineral nutrient stores into the starchy endosperm. Atomic absorption analyses of K, Mg, Ca, Fe, Mn and Zn in grain fractions from both grain types showed small differences, but overall indicate that the *lpa* mutation did not radically alter the mineral nutrient element content compared to the normal Kaybonnet strain.

McKenzie, Ryan, Chatfield, Steve, Ckurshumova, Wenzislava, Ulises, Sergio, Buelna, Sanchez and Berleth, Thomas. Department of Botany, University of Toronto. **Enhancer-trap lines for indirect activation-tagging.**

The plant vascular system is a cellular network that is responsible for the long-distance transportation of substances throughout the plant. An understanding of the molecular cues underlying vascular differentiation will eventually allow for the wood and fibre properties in plants and trees to be manipulated. However, an essential prerequisite for this is the identification and functional characterization of genes that are preferentially expressed in specific stages of vascular development. Making use of an enhancer-trap strategy, we have generated a collection of 6000 transgenic *Arabidopsis thaliana* enhancer-trap lines that express a green fluorescent protein (GFP) reporter and a modified form of the yeast GAL4 transcriptional activator in different temporal and spatial patterns. Here, we show and describe expression patterns that we have observed. We also describe how these enhancer-trap lines can be used to identify genes that are expressed in vascular tissues, and as a means by which the function of other genes can be studied through their mis-expression in the specific vascular cell types marked in each of these enhancer-trap lines.

Tremblay, Reynald, (Colasanti Lab) Department of Molecular Biology and Genetics, University of Guelph. **Functional analysis of *ID-LIKE* genes in *Arabidopsis thaliana*.**

The maize *indeterminate 1* gene, *id1*, encodes a zinc finger protein, ID1, that is a key regulator of the transition to flowering, and also is required for the maintenance of floral development. ID1 is the founding member of the highly conserved ID-LIKE protein family, which is found in all higher plants, including monocots (such as maize and rice) and the dicots (such as *Arabidopsis*). To date, no other *ID-LIKE* gene has been characterized. The *Arabidopsis* genome contains 16 punative *ID-LIKE* genes, 9 of which have been isolated as cDNAs, indicating that these genes are expressed. No mutation correlated with the disruption of an *Arabidopsis ID-LIKE* gene has been reported. This may be the result of genetic redundancy, as seen in studies of other plant gene families, or that loss-of-function *ID-LIKE* mutants may have subtle phenotypes. Sequence alignments of the *Arabidopsis ID-LIKE* genes suggest that gene duplication may have occurred, supporting the possibility of genetic redundancy. We are currently using both public and private gene knockout lines to study the function of the *ID-LIKE* genes found in *Arabidopsis*.

Plant Development Workshops

#	Place Held	Date
1	McMaster University	October 29, 1977
2	University of Western Ontario	April 15, 1978
3	Scarborough College	October 28, 1978
4	University of Guelph	April 7, 1979
5	University of Waterloo	October 20, 1979
6	University of Toronto	March 29, 1980
7	McMaster University	October 20, 1980
8	University of Western Ontario	April 4, 1981
9	Scarborough College	October 31, 1981
10	University of Guelph	April 17, 1982
11	University of Waterloo	November 27, 1982
12	McMaster University	April 4, 1983
13	University of Western Ontario	November 5, 1983
14	University of Toronto	November 3, 1984
15	University of Guelph	May 4, 1985
16	McMaster University	November 9, 1985
17	University of Toronto	April 12, 1986
18	University of Guelph	November 1, 1986
19	University of Western Ontario	April 4, 1987
20	Agriculture Canada - Horticultural Research Institute of Ontario	November 7, 1987
21	University of Waterloo	May 14, 1988
22	University of Toronto	November 5, 1988
23	McMaster University	May 6, 1989
24	Royal Ontario Museum	March 31, 1990
25	University of Guelph	October 27, 1990
26	McMaster University	November 2, 1991
27	University of Montreal	April 11, 1992
28	Trent University	March 20, 1993
29	University of Toronto	March 26, 1994
30	University of Waterloo	April 8, 1995
31	University of Western Ontario	November 16, 1996
32	University of Montreal	October 10, 1998
33	University of Guelph	October 16, 1998
34	University of Toronto	November 4, 2000
35	Wilfrid Laurier University	November 3, 2001
36	University of Western Ontario	November 16, 2002
37	McMaster University & Royal Botanical Gardens	November 1, 2003